Microstructural adaptation of *Zonocerus variegates* (L.) (Orthoptera: Pyrgomorphidae) gut for food storage, digestion and absorption

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Abstract: Micromorphological examination of the gut wall of *Zonocerus variegatus* has shown variations in the wall structure of the crop, midgut and hindgut. The crop is folded longitudinally when empty. It is lined luminally by a cuticular layer. Behind this layer there are small polyhedral cells made into clusters by the longitudinal fold. Three to five fat bundles of striated muscles surround the crop. The midgut which consists of anterior gastric caeca, posterior gastric caeca and ventriculus is cuticle free. Its epithelium is of columnar cells with brush borders. The midgut cells were classified into regenerative, young, resting, extruding and erupting cells depending on the characteristics of their nuclei. The resting cells were the most abundant (42.59%). Anterior gastric caeca, posterior gastric caeca and ventriculus had similar cells. There was a significant difference in the thickness of the epithelia of the six nymphal stages ($P < 0.05$), being thinner at first and second instar nymphs than third, fourth and fifth. The mean thickness of epithelia of sixth instar nymphs observed at 2 day intervals were the same. The hindgut has cuticular layer too, but the underlying epithelium is of columnar cells. The hindgut cells are therefore presumed to help in digestion and absorption of food. Storage, digestive and absorptive functions performed in the pre-hindgut region are suggested to be summarised by the hindgut activities.

Key words: *Zonocerus variegates*; gut; microstructural adaptation; food storage; digestion; absorption

1 INTRODUCTION

The grasshopper, *Zonocerus variegatus*, commonly referred to as the native African grasshopper (Kaufmann, 1965; Modder and Singh, 1976; Modder, 1978), is a polyphagous pest. Its spread is mostly in West Africa as well as in Central and East Africa (FAO, 1989). The omnivorous and gregarious feeding habits of this insect made Lamborn (1914) consider it the worst pest.

Z. variegatus has strong affinity to cassava (*Manihot*) (Chapman *et al*., 1986), which is one of the main sources of carbohydrate in the tropics despite cassava's hydrogen cyanide (HCN) content. The adaptation of the insect to this poisnous substance (HCN) is not certain (Chapman *et al*., 1986) and Belloti *et al*. (1999) reported that this adaptation was based on mere tolerance.

This therefore led to the study of the gut micromorphology to get more satisfactory information on adaptation of the insect to HCN. It is presumed that a closer study of the cells could reveal some structural modifications that could have some adaptation values to the insect. In some acridids, e.g. *Schistocerca*, pockets have been identified in the posterior gastric caeca for the sequestering of toxic substances and no such pocket was observed in *Z. variegatus* (Bernays, 1981).

2 MATERIALS AND METHODS

2.1 Collection of insects and preparation of sections

Adult male and female *Z. variegatus* were collected from IITA, Ibadan, Nigeria. The insects were dissected, guts removed and fixed. The fixed guts were dehydrated, cleared in xylene and impregnated. The impregnated tissues were embedded and sectioned. The sections were stained with haematoxylin and counter stained with Eosin, dehydrated, cleared and mounted. Sections were observed under light microscopes at 10 × and 40 × magnification.

2.2 Determination of epithelial thickness

2.2.1 Thickness of epithelium in the six instar and adult midgut: Sections of the six nymphal and adult stages of the insect were prepared according to histological procedures. Measurements of thickness were done with eyepiece micrometer on light microscope at 40 ×.

2.2.2 Thickness of epithelium between moulting (Day zero) and premoulting (stage termination) period: This insects were bred in the laboratory with fresh cassava whose petioles were kept in water. The

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insects were allowed to moult into the sixth instar. Specimen insects were picked at moulting (Day zero) and subsequently at 2 day intervals, post moulting, until day 22 when adults emerge.

3 RESULTS

Sections of gut regions observed showed different types of cells and arrangements.

3.1 Crop

The crop is surrounded by 3 – 5 fat bundles of striated muscles. Crop lumen has a non-cellular lining which is thrown into folds. Between the muscle pack and the luminal coat are small polyhedral cells, most of which are packed within the folds (Fig. 1: 1).

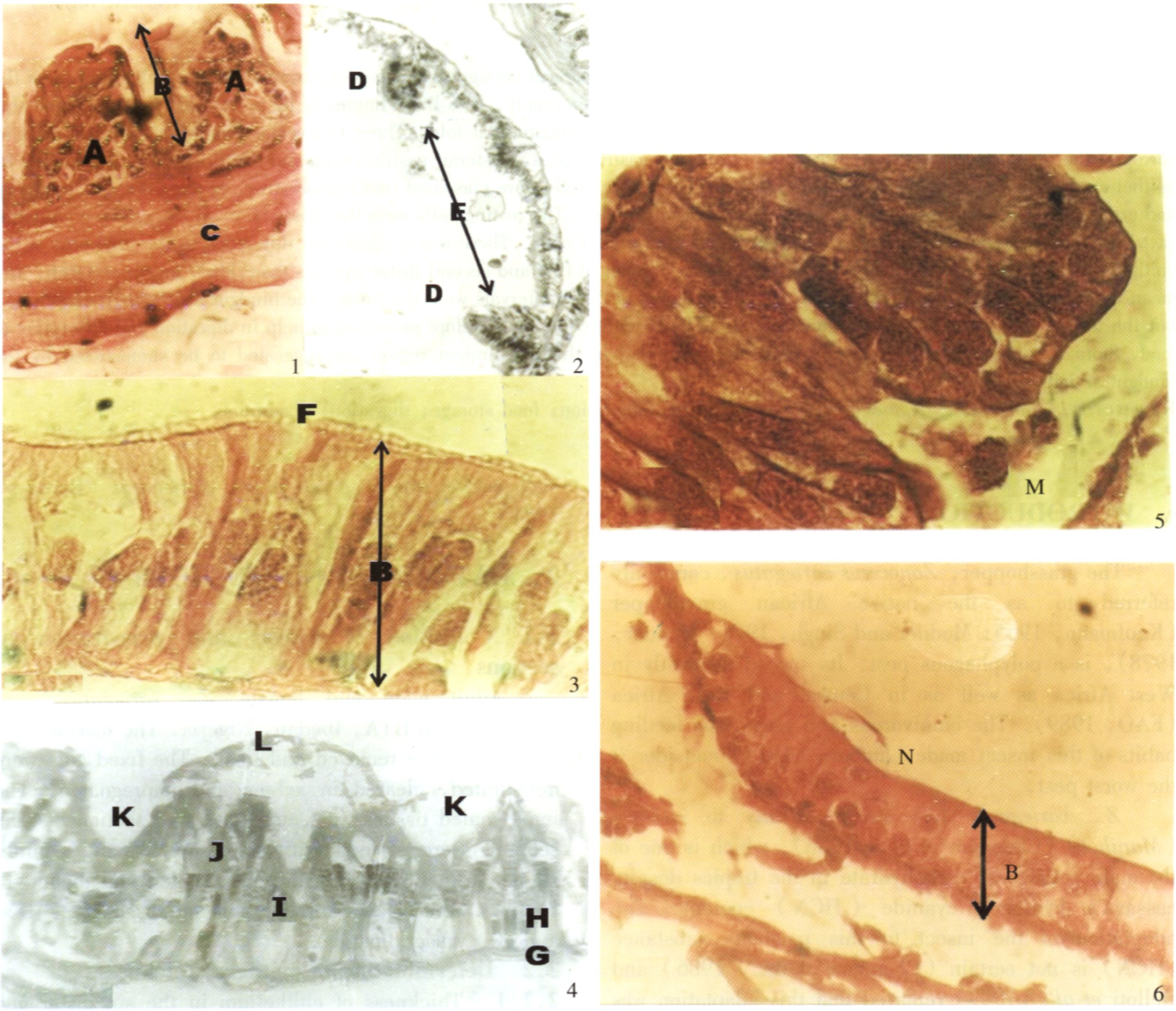

Fig. 1 Sections of the gut of *Zonocerus variegatus*

1. Crop: A: Polyhedral cells; B: Epithelium; C: Muscle bundles. 2. Anterior caeca: D: Fold; E: Crypt. 3. Ventriculus: F: Microvilli (brush border). 4. Posterior caeca: G: Regenerative cells; H: Young cells; I: Resting cells; J: Extruding cells; K: Pocket; L: Peritrophic membrane. 5. Posterior caeca: M: Erupting cell nucleus. 6. Hindgut: N: Cuticular layer; B: Epithelium.

3.2 Midgut

The midgut, which consists of anterior gastric caeca, posterior gastric caeca and ventriculus, shows the same microstructures.

3.2.1 Anterior gastric caeca: The caeca observed under light microscope consists of a simple layer of columnar cells surrounding a simple cavity. This epithelium rests on a muscle layer. The epithelium varies in thickness between 2.5 and 8.0 μm. This is due to varying extension of luminal cytoplasm of the columnar cells. The epithelium is thrown into folds（about 3.2 μm high）（Fig. 1：2）. The folds are separated from one another by crypts. The shape and size of deeply stained nuclei gradually changed from small ovoidal ones at the top of the fold to large spherical ones at the crypts.

The columnar cells have striated border（Fig. 1：3）. Vesicles were observed on cell surfaces. Cell boundaries were vague. Identification was done with nuclei. The apical cytoplasm was denser containing more granules than the basal cytoplasm of the cells. The nuclei vary in shape，size，position and staining. Based on these variations，the epithelial cells were categorised as regenerative，young，resting，extruding and erupting cells（Fig. 1：4，5）. Among these cells，the resting cells were most abundant（18.59 ± 5.05 per unit area）（42.59%）. This was followed by the regenerative cells（13.17 ± 5.92）（30.18%），extruding cells（7.41 ± 6.76）（16.99%），young cells（3.27 ± 2.87）（7.47%）and erupting cells（1.00 ± 6.00）（1.20%）.

ⅰ. Regenerative cells：These cells occur mostly beneath the columnar cells. Unlike most regenerative cells they do not restrictively occur at the bases between the columnar cells. Their nuclei are small and of various shapes which may be elongated or polygonal. They occur singly or in a small group（nidi）of 2 – 10 cells.

ⅱ. Young cells：The cells shoot out from regenerative nidi. Their nuclei are small elongated and densely pigmented. Their nuclei are located a little above the regenerative cell's nuclei.

ⅲ. Resting cells：They are cells with oval nuclei. The chromatin materials are heavily pigmented but not much clustered. Their nuclei appear light and heterogeneous. Their nuclei are above the nuclei of the young cells. Their brush border is clear and distinct.

ⅳ. Extruding cells：These cells have elongated nuclei which are larger than the young cell's nuclei. Their nuclei have blunt luminal ends and tapering basal ends which give them“club”or pestle appearance. In some the basal ends are lighter and appear vacuolated. Their nuclei are heavily pigmented，giving a dark staining appearance. Most of these cells' nuclei are at levels above the nuclei of resting cells. Brush border may be present.

ⅴ. Erupting cells：These are already degenerated cells. They could be identified by erupted nuclei or fragments. Their luminal surfaces are broken and the brush border disappears.

3.2.2 Posterior gastric caeca：The posterior gastric caeca are similar to the anterior gastric caeca in cell types and epithelial thickness. The posterior gastric caeca is indented by tiny pockets（Fig. 1：4，5），which are between 0.3 and 1.0 μm deep and vary in width between 1 and 5 columnar cells. The pockets are simple pits whose floors are formed by cells with shorter luminal cytoplasms，and the walls by extending lateral walls of the bordering cells. These pockets are neither localised nor branched，but are randomly distributed（about 12 pockets per 10 μm long）.

3.2.3 Ventriculus：Ventriculus was also lined with a layer of columnar cells. Cells present in anterior and posterior gastric caeca are found in the ventriculus.

3.2.4 Estimation of midgut thickness：

ⅰ. The mean thickness of the epithelia in anterior gastric caeca and ventriculus for the six nymphal stages and adult is shown on Table 1. There was a significant difference in the thickness of the epithelial between all the development stages（$P < 0.05$）.

ⅱ. The mean thickness of epithelia in sixth instar nymphs observed at 2 days intervals are presented on Table 2. There was no significant difference between the epithelia thickness of the various day intervals.

Table 1　Means midgut thickness in different larval stages and adult of *Z. variegatus*（mm）

Gut regions	Stages						
	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ	Adult
Anterior caeca	0.37 ± 0.08	0.37 ± 0.08	0.55 ± 0.09	0.40 ± 0.04	0.40 ± 0.04	0.51 ± 0.09	0.54 ± 0.12
Posterior caeca	0.34 ± 0.07	0.29 ± 0.04	0.55 ± 0.09	0.47 ± 0.07	0.45 ± 0.09	0.49 ± 0.05	0.71 ± 0.18
Ventriculus	0.36 ± 0.05	0.32 ± 0.06	0.60 ± 0.05	0.53 ± 0.05	0.43 ± 0.07	0.49 ± 0.07	0.67 ± 0.09

Notes：Figures are mean ± *SD*（$N = 20$）（$P < 0.05$）.

Table 2　Midgut thickness of Ⅳ instar of *Z. variegatus* at 2 day intervals from moulting（Day 0）to premoulting（Day 22）（mm）

Gut regions	Days after moulting							
	0	2	4	6	8	12	18	22
Anterior caeca	0.48 ± 0.08	0.45 ± 0.04	0.47 ± 0.10	0.44 ± 0.06	0.45 ± 0.05	0.47 ± 0.06	0.43 ± 0.06	0.48 ± 0.02
Posterior caeca	0.58 ± 0.08	0.44 ± 0.03	0.53 ± 0.04	0.58 ± 0.17	0.41 ± 0.15	0.46 ± 0.10	0.55 ± 0.05	0.39 ± 0.09
Ventriculus	0.58 ± 0.10	0.55 ± 0.15	0.48 ± 0.04	0.42 ± 0.10	0.36 ± 0.05	0.48 ± 0.03	0.48 ± 0.06	0.30 ± 0.10

Notes：Figures are mean ± *SD*（$N = 20$）（$P < 0.05$）.

3.3 Hindgut

A section of the hindgut (Fig. 1:6) shows a layer of columnar cells on a basement layer. The cells have round nuclei which locate near the basal surface. The apical surfaces of the cells have a covering which is represented by the letter N on the plate. The cells boundaries are less vague.

4 DISCUSSION

4.1 Crop

The structure of the crop wall consisting of muscle blocks, polyhedral cells and folded cuticular coating helps it to carry out its traditional function of temporary storage and regulation of food passage (Treherne, 1967; Guthrie and Tindal, 1968; Turunnen, 1985). The muscle blocks enhance full crop distension without tearing. The polyhedral shape of crop cells probably allows alterations (without cell damage) in the size of the crop, a function which cell with definite shape could not perform. The position of the cells between the muscle bundles and cuticular layer is suggested to be advantageous by purportedly preventing interference and subsequent obstruction of fold stretching by the muscle bundles during distension. The crop is intact and completely impermeable to even small molecules (Treherne, 1967) and is consistent with it storage function (Berridge, 1969).

4.2 Midgut

The midgut epithelium is a layer of columnar cells, on a basement layer over a muscle layer (lesser in thickness than crop muscles) as in most insects (Khan, 1964; Gouranton, 1968; Smith *et al.*, 1969). The thinness of the epithelium probably enhances quick passage of nutrients into the haemolymph. The folds and crypts on caeca surfaces are devices suggested to increase the caecal area for digestion and absorption. The crypts of the posterior caeca are assumed analogs of the folds of anterior caeca. In addition, the crypts allow the concentration of solutes to enhance sequestering of wastes and passage of water into the haemolymph (Bernays, 1981).

The clear brush border observed on the surface of the midgut epithelia is suggested to be associated with digestion and absorption of food. This is because most digestion and subsequent absorption take place at muccopolysaccharide matrices (brush border) of the cells (Smith *et al.*, 1969).

The cells pass stages of development moving upwards from the interstitial nidi to be structurally and functionally matured, degenerate and be replaced by new cells below. The cells in the resting and extruding phases probably perform more digestive functions than absorption or *vice versa* as characterised by the nature of their nuclei. In the young phase, the cells are presumed to be newly formed by the interstitial cells. These new cells then migrate into the epithelium proper to occupy their positions.

As they migrate, their free border of luminal surface develops striations or microvilli. On arriving at the epithelium proper, they settle and grow to full size. Similar observations had been made by Khan (1964). This growth gives more space for the nuclei to stretch. Hence the chromatin materials are not clustered as in other cells. Although these nuclei contain heavily stained chromatin materials, their nuclei look more dispersed. Also since the cells appear steadier at this stage, their nuclei assume a more central position in the cytoplasm than others. These probably account for the oval shape of the resting cell nuclei.

In the extruding phase, the cells are suggested to shift position luminally. During the shift, probably under the pressures from the bordering cells, their chromatin materials become congested, giving the nuclei homogenous dark appearance. Since the nuclei are extruding in the direction of the cell shift, these nuclei become elongated with blunt anterior end while the posterior end from which it shifts tapers, giving the nuclei a“ club ”shape. Since the shift is apical, the position of the nuclei is thus above the level of resting cells nuclei. Most of these cells contain very conspicuous vacuoles on top of their nuclei (Fig. 1:4) which are presumed to contain autophagic materials. Much of the organelle breakdown probably is done at the extruding phase of the cells, by then the cells might be old enough to be replaced. So autolysis can start before the cell enters the final stage. The broken materials will be discharged as secretions to be re-ingested.

The erupting cells are the old cells that presumably degenerate after discharging their autophagic contents into the lumen. They are identified by their nuclei sticking out above the epithelium (Fig. 1:5). The nuclei remain the last structure to be broken down probably because of its role to initiate the breakdown of other organeles.

In general these epithielial cells show much irregularity as to the height of the nuclei. Actively secreting cells are much higher than older cells (Owsley, 1946). Probably their secretions contain digestive enzymes that qualify them as active digesting cells (Saxena and Chads, 1971). Furthermore, a decrease in chromatin size indicates increased cellular activities (Jangueira *et al.*, 1977) confirming the cells to be more active than the other cells.

From these observations, it could be suggested that the midgut epithelium, like most insect epithelia, is continually renewed. And the cells pass through absorptive and secretory phases before degenerating（Wigglesworth，1967）.

The secretory activity of the epithelium could not be clearly defined as merocrine or holocrine，because the cells which are digestive and absorptive are passing through different life phases before they disappear. Phytophagous insects are mostly merocrine（Pradhan，1940）and omnivorous species are of the holocrine types（Owsley，1946）. Some insects have both，which is presumed to represent the various stages in cellular degeneration（Tschang，1929）. Both types could be concluded to occur in *Z*. *variegatus*.

Secretory activities among the various instars observed were different. This depends on the feeding intensities of the various larval stages. In the fifth and sixth instars where feeding is high（Modder and Singh，1978；Akpan and Okorie，2003），the secretory activity results in the observed differences in the thickness of the epithelia. Within each instar，secretory activity remains the same throughout the duration of the stage especially in the larval stages.

4.3 Hindgut

The hindgut epithelium is of columnar cells with striated border. The luminal surface is lined with cuticular covering which prevents mechanical damage of the epithelial cells by faecal materials. Since it is perforated it serves additional sieving function at the rectum（Phillips and Dockrill，1968）.By possession of brush border cells，hindgut must be secreting digestive enzymes into the stored material for miscellaneous digestion and simultaneously absorbs nutrients and water from the material. The cuticular lining of the hindgut surface is different from that of the crop by having perforations（Klein and Applebaun，1975）through which cells underneath can resorb water. It is also reduced in thickness over the pads and sometimes separated from the principal cells of the rectal pad（Phillips *et al*.，1986）. The permeability properties of rectal cuticle have been fully studied by Phillips and Dockrill（1968），Phillips and Beaumont（1971），and Phillips *et al*.（1986）. Rectal resorption permits recovery of water，the major monovalent ions and basic metabolites of amino acids secreted by the Malpighian tubules（Phillips *et al*.，1986）. The main function of storage and digestion/absorption served by crop and midgut respectively are summarized in the hindgut which stores，digests and absorbs food materials. The insect gut is simple but properly designed for effective feeding processes as in complex and higher animals.

The observations made to far do not indicate any adaptative strategy of the insect to the hydrogen cyanide of the cassava plant.

References

Akpan BE，Okorie TG，2003. Allometry growth and performance of the gastric caeca of *Zonocerus variegatus*（L.）（Orthopters：Pyrgomorphidae）. *Acta Entomol*. *Sin*.，46（5）：558 – 566.

Bellotti AC，Smith L，Laponite SL，1999. Advance in cassava pest management. *Ann*. *Rev*. *Ent*.，44：343 – 370.

Bernays EA，1981. A specialized region of the gastric caeca in the locust，*Schistoerca gregaria*. *Physiol*. *Entomol*.，6：1 – 6.

Berridge MJ，1969. A structural analysis of intestinal absorption. In：Insect Ultracstructure. *Roy*. *Entomol*. *Sco*. *Symp*.，5：135 – 151.

Chapman RF，Page WW，Mccaffery AR，1986. Bionomics of the variegated grasshopper *Zonocerus variegatus* in West and Central Africa. *Ann*. *Rev*. *Ent*.，31：479 – 505.

Food and Agricultural Organization（FAO），1989. A record of the activities of the FAO locusts and other migratory pest and emergency of operation group. *Migrant Pest Newsletter*，57 – 58：1 – 29.

Guthrie DM，Tindal AR，1968. The Biology of the Cockroach. Edward Arnold Ltd. London. 260 – 286.

Gouranton J，1968. Observations histochemiques et histoenzymiologiques sur Ie tube digestiti de quelques（Homoptera：Oeropiedes et Jassides）. *J*. *Insect Physiol*.，14：569 – 579.

Janqueira IC，Carneiro J，Contoponlos A，1977. Basic Histology（2nd Edition）. Los Altos，California：Lange Medical Publications.

Kaufmann T，1965. Observations on aggregation migration and feeding habits of *Zonocerus variegatus* in Ghana（Orthoptera：Acrididae）. *Ann*. *Entomol*. *Soc*. *Am*.，58：226 – 436.

Khan MA，1964. Histological changes related to the secretion of digestive enzymes in the midgut and caeca of *Locusta migratoria* L.（Orthoptera：Acrididae）. *Proc*. *Roy*. *Entomol*. *Soc*. *Lond*.（A），39（7 – 9）：118 – 124.

Klein M，Applebaun SW，1975. The surface morphology of locust hindgut cuticle. *J*. *Ent*.（A），50（1）：31 – 36.

Lamborn WA，1914. Agricultural pest of the southern provinces，Nigeria. *Bull*. *Entomol*. *Res*.，5：197 – 214.

Langewald J，Thomas MB，Douro-Kpindou OK，Lomer CJ，1997. Use of *Metarhizium flavioride* for control of *Zonocerus variegatus*. A model，linking dispersal and secondary infection from the spray residue with mortality in cage field samples. *Entomol*. *Exp*. *Applic*.，82：1 – 8.

Modder WWD，1978. Respiratory and weight changes and water uptake during embryonic development and diapause in the African grasshopper，*Zonocerus variegatus*（L.）（Acrididae：Pyrgomorphidae）. *Acrida*，7：253 – 265.

Modder WWD，Singh SR，1976. Utilization of cassava，*Manihot esculenta* in the laboratory by the later instars of the African grasshopper，*Zonocerus variegatus*（L.）（Acrididae：Pyrgomorphidae）. *Rev*. *Zool*. *Afri*.，90（2）：417 – 430.

Owsley WWB，1946. The comparative morphology of internal structures of the Asilidae（Diptera）. *Ann*. *Entomol*. *Soc*. *Am*.，30：33 – 85.

Phillips JE，Dockrill AA，1968. Molecular sieving of hydrophilic molecules by the rectal intima of the desert locust，*S*. *gregaria*. *J*. *Exp*. *Biol*.，48：521 – 532.

Phillips JE，Beaumont A，1971. Symmetry and non-linearity of osmotic flow across rectal cuticle of the desert locust. *J*. *Exp*. *Biol*.，54：317 – 328.

Phillips JE，Hanrahan J，Chamberlain M，Thomas B，1986. Mechanisms of control of reabsorption in insect hindgut. *Adv*. *Insect Physiol*.，19：329 – 422.

Pradhan S，1940. The alimentary canal and proepithelial regeneration in *Coccinella septempunctata* with a comparison of carnivorous and herbivorous coccinemds. *Quart*. *J*. *Microscope Sci*.，81：451 – 478.

Saxena PN，Chads HL，1971. The green bug *Schizaphis graminum* digestive system. *Ann*. *Entomol*. *Soc*. *Am*.，64：1 031 – 1 038.

Smith DS，Compiler K，Janness M，Lipton C，White LW，1969. Cellular organization and ferritin uptake in the midgut epithelium of moth，*Ephestia kuhniella*. *J*. *Morp*.，127：41 – 72.

Treherne JE，1967. Gut absorption. *Ann*. *Rev*. *Entomol*.，12：43 – 58.

Tschang YT，1929. L'histogenes et l'mistophysiologie de l'epithelium de l'intestin moyen chez un lepidoptete（*Galleria mellonella*）. *Bull*.

Biol., 12:1－144.

Turunen S, 1985. Absorption. In:Kerkut GA, Gilbert LI eds. Comparative Insect Physiology and Biochemistry. Vol.4. 241－277.

Wigglesworth VB, 1967. The Principles of Insect Physiology. Methuen, London. 427－495.

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臭腹腺蝗(直翅目:锥头蝗科)消化道对食物储存、消化和吸收相适应的显微结构

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摘要:显微观察发现臭腹腺蝗 *Zonocerus variegatus*(直翅目:锥头蝗科)嗉囊、中肠和后肠的肠壁结构有所不同。嗉囊为空时纵向折叠。中肠上皮层的厚度随龄期有明显变化,1 龄和 2 龄时明显大于 3 龄、4 龄和 5 龄。后肠具有帮助消化和吸收的功能。

关键词:臭腹腺蝗;消化道;显微结构适应;食物储存;消化;吸收

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